PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P223	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/US2005/004497	International filing date *(day/month/year)* 09.02.2005	Priority date *(day/month/year)* 09.02.2004
International Patent Classification (IPC) or national classification and IPC INV. C12N15/62 C12N15/85 C07K14/705		
Applicant SYNAMEM CORPORATION et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.12.2005	12.05.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Hornig, H Telephone No. +31 70 340-2620

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2005/004497

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-22	as originally filed

Sequence listings part of the description, Pages

1, 2	as originally filed

Claims, Numbers

1-13	received on 12.12.2005 with letter of 09.12.2005

Drawings, Sheets

1/5-5/5	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2005/004497

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-5,9
	No:	Claims	1,6-8,10-13
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Re Item 1

1.1 The amended claims 1-13 filed with letter dated 09.12.2005 and received on 09.12.2005 are allowable according to Art. 34 (2)(b) PCT. The basis of the opinion issues on the claims 1-13 as amended acording to Art. 70.2 PCT.

Re Item V.

1 Reference is made to the following documents:

D1 : WO 03/089649 A (OXFORD BIOMEDICA LIMITED; KINGSMAN, SUSAN; CARROLL, MILES; MYERS, KEV) 30 October 2003 (2003-10-30)
D2 : WO 96/41865 A (ARIAD GENE THERAPEUTICS, INC; CLACKSON, TIMOTHY; HOLT, DENNIS, A; GILM) 27 December 1996 (1996-12-27)
D3 : WO 94/18317 A (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIO; PRESIDENT AND FELL) 18 August 1994 (1994-08-18)
D4 : WO 02/061389 A (TANOX, INC. [US]) 08 August 2002 (2002-08-08)

2 INDEPENDENT CLAIMS 1, 8 and 11

2.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 and 8 is not new in the sense of Article 33(2) PCT. Document D1 discloses an expression vector comprising an amino-terminal tag sequence and a signal sequence operably linked to a nucleotide sequence of interest, where the amino-terminal tag sequence is inserted between the signal sequence and the nucleotide sequence of interest which is a tumour associated antigen (TAA 5T4), characterised as membrane protein. Constructs for a membrane-bound protein are made which were cloned in pIRES-STAR vector and transiently transfected into CHO cells and expression of h5T4 detected by immuno-staining of fixed cells with an anti-myc antibody (Examples 1-3, Fig. 1-4).
Therefore, a method of generating tethered extracellular domains of transmembrane

proteins comprising: (a) preparing an expression vector comprising a 5' signal sequence, a purification epitope tag, a sequence coding for the extracellular domain of a membrane protein and a 3' anchor sequence, and transfecting mammalian cells with said expression vector to generate anchor tethered protein targeted to the extracellular domain of a plasma membrane does already exists.

2.2 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 and 11 is not new in the sense of Article 33(2) PCT. Document D2 discloses configurations for biological switches and provides new methods and materials useful for regulating biological events in animal cells. The invention involves recombinant DNA constructs comprising DNA sequences derived from sequences encoding the proteins FRAP, Tor1, Tor2 and other proteins capable of binding to FKBP:rapamycin. The products can be used for regulating biological events such as gene transcription and activation of an intracellular signal transduction pathway. Furthermore D2 describes the cloning of the cytoplasmic domain of a receptor tyrosine kinase into the XbaI site of pCMFR series or pCMF series of vectors and the cotransfection into Cos-1 cells by lipofection (page 100, lines 16-page 101, lines 27).
The plasmids pCMF11/2/3.HA respectively pCMFR1/2/3.Flag have the following features: a myristoylation domain and a HA, respectively a Flag epitope tag and a XbaI site in between, into which the cytoplasmic domain of a receptor protein was cloned.

Therefore, a method of generating tethered extracellular domains of transmembrane proteins comprising: (a) preparing an expression vector comprising a 5' myristoylation encoding sequence, a sequence coding for the intracellular domain of a membrane protein and a 3' purification epitope tag, and transfecting mammalian cells with said expression vector to generate myristoylated tethered protein targeted to the intracellular domain of a plasma membrane does already exists.

2.3 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 and 8 is not new in the sense of Article 33(2) PCT. D4 describes a method of generating monoclonal antibodies to a large number of mammalian antigens comprising cloning gene fragments from a genomic or a cDNA library into a fusion vector having a promoter sequence, a signal peptide sequence, a cloning site, and a binding region sequence specific for an antigen presenting cell

membrane receptor, and transducing or transfecting immature antigen-presenting cells with the vector library. Moreover, D4 discloses the cloning monoclonal antibody gene fragments used in the novel method into a display vector comprising a promoter sequence, a signal sequence, an epitope tag, a cloning site, and a transmembrane domain sequence. Furthermore, D4 teaches the purification of heterologous protein and peptide moieties using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, c-myc, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, c-myc, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags

3 DEPENDENT CLAIMS 2-7, 9-10 AND 12-13

Dependent claims 2-5 and 9-13 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT).